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(54) Title: APPARATUS AND METHOD FOR DETECTING HEMAGGLUTINAION REACTIONS

(57) Abstract

There is disclosed an apparatus and method for doing pattern recognition processing of the digitized image of well bottoms or other material containing light and dark images which contain information being sought in an assay or other operation such as DNA sequencing. The light and dark patterns are compared to stereotypical patterns of light and dark in known assay results or other known results to determine a type for an experimentally determined image. In the blood typing and grouping field of use, the pattern of positive and negative reactions to blood typing reagents used in the forward and reverse typing procedures is then compared to known patterns to derive the blood type and group of the donor.

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# APPARATUS AND METHOD FOR DETECTING HEMAGGLUTINATION REACTIONS

## BACKGROUND OF THE INVENTION

The invention pertains to the field of processing the results of chemical assays. In particular, the invention pertains to the field of analysis of the light reflectance properties of patterns of hemagglutination reactions between donor cells and chemical reagents such as are used in antibody testing and blood grouping. The invention also pertains to characterizing reactions between donor plasma and known reagent cells (backtyping in the blood typing art) or for characterizing reactions in antibody screen testing or infectious disease testing.

There currently exist machines called plate readers which are used to identify the pattern of positive and negative reactions between donor cells and certain chemical reagents used for antibody testing and blood grouping. Typically, in blood group and type assays, donor red blood cells and donor plasma are placed in two groups of transparent assay wells. Then a series of different reagents are added to the wells. If a positive reaction occurs in any well, hemagglutination results.

Hemagglutination is the process of binding of the chemical reagent to donor cells to form clumps of cells which fall to a tight pellet at the bottom of the assay well when they are spun in a centrifuge. Thus, a positive reaction will be characterized by the presence of these clumps of cells in the bottom of the assay well. A negative reaction will not result in large clumps of cells being formed which fall to the bottom of the test well. The pattern of positive and negative reactions to the various reagents determines what ABO group and type of blood the donor cells came from. Similar techniques apply in the field of antibody screening tests.

Current plate readers use light absorbance data from shining a light beam through the bottom of the well to determine if a particular well has had a positive reaction or a negative reaction. This is done by shining a light beam through the well bottom on at least two different paths and measuring the light absorbance on each path. For positive reactions in test wells that have smooth U shaped bottoms, the clumps of cells are compacted to the center. Swirling the test wells will not break apart these clumps and a "button" of cells will appear in the well at the lowest point of the well which is usually on the centerline. Swirling negative reactions will cause a diffuse, relatively homogenous mixture. Typically, the plate reader will shine a beam of light along a path

passing outside the region that buttons will appear. The positions that the plate reader is set to can be simply stated as "off center". If a negative reaction has occurred, the light absorbance reading will indicate much less light has reached the sensor along the path through the location outside the button. If a positive reaction has occurred such that a "button" has formed, the light absorbance readings along the path will be very low.

A problem with current plate readers is that for non U-shaped bottom assay wells such as the wells in plates made by Olympus, "buttons" do not always form in the bottom center of the well. The reason for this is that such well bottoms are "terraced" in that the bottoms of the well slope to a very small flat spot in the bottom middle of the well via a series of stair steps or plateaus along the sloping bottom. These terraces have flat upper surfaces. These plateaus tend to catch the "clumps" of proteins as they drift down and come to rest on the tops of the steps. Because the tops of the steps are flat and horizontal in normal assay position, gravity does not tend to pull the hemagglutination clumps toward the middle of the bottom of the well thereby preventing the formation of a button even when a positive reaction has occurred. Thus the bottom of such a well with a positive reaction will have agglutination clumps covering the entire bottom surface outside the flat area in the middle and will have

scattered clumps of agglutinated cells scattered randomly throughout the center flat surface as well. Thus, instead of a button of agglutinated cells being formed, the entire well bottom in such terraced wells will be covered with agglutinated cells. Further, because of the terraces and the sharp edges in the plastic of the well bottom which define the terrace steps, the light in the light beam which is not passed through the well bottom center is scattered by the terrace steps. Thus, a conventional plate reader attempting to read such a well in a conventional manner might misinterpret the light absorbance pattern of a positive reaction for the light absorbance pattern of a negative reaction by getting low light absorbance readings on both paths. Further, the use of precisely located light beam paths in conventional plate reader technology places a requirement that the plate position in the plate reader be very precisely controlled so that the path that the light beam takes through the bottom of the well is predictable.

This tight positional control requirement for the plate position is even more pronounced in the case of terraced plates. The reason is that in the terraced plates, the center flat spot with no terraces is very small. In fact, the center flat spot is approximately the same size as the light beam in the plate reader itself. Because just outside this flat spot, the terraces start

and the optical characteristics change dramatically, it becomes quite difficult to use conventional plate reader technology to distinguish positive and negative hemagglutinations from plate changes.

One method of circumventing some of the above noted problems is to take many readings using the offset light beam method. The problem with this approach is that it takes too much time. Currently, every known spectrophotometric plate reader which operates according to the above noted principle takes about 8-10 minutes to read one plate of 8 samples with 16 readings per well. To increase the number of readings would substantially increase the time to read a plate and still would not yield accurate results for terraced plates although it would lead to more accurate results for U-shaped bottoms.

A need has thus arisen for a method and apparatus which can accurately read the light absorbance patterns of both positive and negative reactions for any kind of assay well bottom configuration without the tight positional control on the plates necessary in prior art plate readers.

## SUMMARY OF THE INVENTION

According to the teachings of the invention, there is disclosed herein a method and an apparatus for digitizing the light intensity pattern of light reflected

from the bottom of a plurality of wells in which assays have been carried out. Pattern recognition is then carried out on the light intensity pattern based upon the type of well bottom type which gave rise to the light intensity patterns. This pattern recognition defines whether the reaction in each well was positive or negative depending upon the light intensity pattern in the well.

In the preferred embodiment, the apparatus is comprised of an IBM AT CPU driving a flat bed scanner. The flat bed scanner scans and digitizes the light absorbance characteristics of the bottoms of the wells and sends this data to the CPU for processing. The CPU then processes the data by reading a configuration file to determine what type of wells are in use. Next the data is retrieved for the first well bottom image and reconstructed to its uncompressed format. A branch is then made to either of two pattern recognition subroutines depending upon the condition of a variable set from the data from the configuration file or user input defining the type of well bottoms in use.

If terraced well bottoms are in use, the CPU averages the light intensity values of the pixels in the center region where the flat spot occurs and compares this average to the average light intensity for the pixels outside the center flat region of the well bottom. If the averages are substantially different, the well

identification number is stored along with data indicating the reaction in the well was negative. If the averages are not substantially different, the well identification is stored along with data indicating that the well reaction was positive. Processing then returns to a point in the program where the pixel data for the next well to be analyzed is retrieved. The above noted pattern recognition process then repeats itself.

If the well bottom is U-shaped, the pixel data for the well bottom center is averaged and compared to the average for the region outside the center of the well. If the averages are substantially different, the well identification is stored with data indicating that the reaction is positive. If the averages are not substantially different, the well identification is stored with data indicating that the well reaction is negative. Processing then returns to a place in the program to retrieve the pixel data for the next well to be examined.

In an alternative embodiment, after retrieving the data and decompressing it, a branch to the proper well bottom shape pattern recognition routine is made. For terraced bottoms, each pixel is compared to its neighbors. If there is a large difference for one or more of the comparisons or any number of comparisons above a given threshold, then the well reaction is determined to be negative. Otherwise, it is determined to be positive

since no sharp contrast edge appears in the optical absorbance data appears. If a U-shaped bottom is in use, the same comparison process is used, but a large difference in one or more of the comparisons indicates that a positive reaction has occurred whereas the absence of a large difference indicates that a negative reaction has occurred.

After all the wells have been so processed, the well data for each donor sample, i.e., the reaction template for that donor, is compared to known templates for the various blood types or assay results which are possible. If there is a match with any known template, that donor identification record in a data base is updated with the blood type and group or with the results of the particular assay which was performed. Note that the above defined apparatus and method may be generalized for any use which creates a pattern of light and dark areas of light absorbance in a pattern which convey information. The computer may determine the pattern of light and dark area and compare this pattern to a group of known patterns. Such a machine can find use in may different antibody assays and in infectious disease testing.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a block diagram of the hardware of the computer system which implements the invention.

Figure 2 is a digitized image of a plurality of terraced well bottoms some of which have experienced positive agglutination reactions, some of which have experienced negative agglutination reactions and some of which are empty.

Figure 3A is a diagram of a terraced well bottom showing what happens to the agglutination particles during a positive reaction as they are centrifuged out of solution.

Figure 3B is a diagram of a U-shaped well bottom showing what happens to the agglutination particles during a positive reaction as they settle out of solution.

Figures 4A and 4B are a flow diagram illustrating a typical pattern recognition process which could be used to implement the teachings of the invention in the field of blood typing and grouping.

Figure 5 is a flow diagram of the process of matching the pattern of positive and negative agglutination reactions to a plurality of known patterns of positive and negative agglutination reactions to the same reagents for known blood types.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring to Figure 1, there is shown a block diagram of a computer system which implements the preferred embodiment of the invention. The invention can

be implemented in any computer 10 that is capable of driving a flat bed scanner 12 and which has the memory capacity to store the digitized data generated by the flat bed scanner 12. In the preferred embodiment, the computer 10 is an IBM AT with 2 megabytes of RAM and a 70 megabyte hard disk. This much capacity is not necessary however. It is only necessary that the computer 10 have sufficient hard disk storage to store enough pixel data from the flat bed scanner 12 to define one plates worth of well bottom's light reflectance data. Generally, this is about 1 megabyte of pixel data. Further, the computer 10 must have sufficient RAM to run the driver software for the flat bed scanner 12, the operating system, and the pattern recognition program to be described below. Generally, an IBM AT with 640K of RAM and a 20 megabyte hard disk will be adequate for purposes of practicing the invention.

In the preferred embodiment, the flat bed scanner is a Data Copy Scanner which is commercially available. This scanner comes with a driver board which is coupled to the data, address and control buses of the computer 10 via an expansion slot. There is also driver software which comes with the flat bed scanner called PC Image.

The flat bed scanner works in a manner which is well known to those skilled in the art and is similar to making a photocopy. Basically, the scanner includes a

light bar (not shown) which moves along under a glass plate 14 similar to that found on well known copy machines. The light from the light bar is projected against the bottom of a plate 16 of test wells including wells 18 and 20. The image of the bottom of the wells of plate 16 contains light and dark area depending upon the optical properties of the wells and upon the type of reactions that have occurred in each well. The image of the bottoms of the wells is digitized by the flat bed scanner 12, and the data is output to the CPU 10 on the bus 22. The CPU 22 stores this digitized image of the well bottoms on a hard disk (not shown). The CPU 10 is controlled through a keyboard 24 and displays messages to the operator by a monitor 26.

Referring to Figure 2, there is shown a representation of the actual light and dark patterns in a digitized image of the bottoms of a plurality of test wells. Some of these test wells have donor samples which have experienced positive reactions, and some of the test wells have donor samples which have experienced negative reactions. Some of the test wells are empty. The wells labeled with reference numbers 28, 30 and 32 contain donor material which has experienced negative reactions with reagent material added to the wells, i.e., no agglutination has occurred. The test wells labeled 34 and 36 have donor samples which have experienced a positive

reaction with reagent material added to these wells, i.e., agglutination has occurred. All the other test wells in Figure 2 such as well 38 are empty. Note that it is easy to distinguish positive reaction wells from negative reaction wells by the digitized images in that the negative reaction wells (all wells in Figure 2 are terrace bottomed) have a dark spot in the center of the well and a lighter field surrounding this dark spot. On the other hand, the positive reaction wells have a uniform lighter shade throughout the area of the bottom of the well. The empty wells have randomly scattered sectors of lighter and darker areas in the shape of pie segments extending from the circumference of the well to the center. In U-shaped bottom wells, the situation is exactly reversed. A key feature of the method and apparatus of the invention is to be able to distinguish between these various empty, positive and negative wells by the shapes and locations of the light and dark spots in the digitized image of the bottom of each assay plate. To that end any pattern recognition software which can make such distinctions and identify the wells as they have been identified in Figure 2 will suffice for purposes of practicing the invention.

Figure 3A shows a terraced bottom shape such as is found in the Olympus microtitre plate. The particles such as the particle 40 which are shown as falling out of solution represent agglutinations between donor cells and

the reagent chemical or cells added to the well. These particles are shown as falling out of solution toward the bottom of the well. If a negative reaction had occurred, no such particles would exist, and the solution of donor cells or plasma plus reagent would have equal light absorbance throughout its volume. Note because of the terrace steps having flat tops which are normal to the gravity vector, the agglutination particles come to rest on the bottom and stay where they fall since there is no slope toward the center on the top of any particular step. Thus, the entire bottom of the well becomes coated with particles thereby creating a uniform film of light absorbing material. If light is directed toward the entire bottom surface 42 and the image of the bottom surface 42 is digitized, the image will have a uniform light absorbance property throughout its area. Note that one of the reasons why reading light absorbance of individual conventional light beams as is done in plate readers is not accurate is apparent from study of Figure 3A. In a conventional plate reader, a light beam 44 would be directed through the substantially flat center section 46 of the plate for a first reading. The light beam would pass through the center section on a straight path unless it was blocked by a film of agglutination particles resulting from a positive reaction. The amount of light intensity in the light beam after it passed through the

bottom would then be measured. The light beam would then be deflected to pass through the bottom on a second path shown at 48. This path is set to not pass through the center of the well bottom. Note that this light beam gets scattered as symbolized by the beam components 50 and 52 as it passes through the bottom of the well because of the sharp edges in the material of the well bottom caused by the terraces. Figure 3A is not drawn to scale, and, in reality, there are hundreds or thousands of terrace steps in the bottom of each well. This scattering would be interpreted falsely as light absorbance even though there may be no particles resting on the bottom of the well from a positive reaction.

The light scattering in the region outside the area of the center flat section of Figure 3A defined by the diameter D creates the distinctive patterns of light and dark regions shown in Figure 2 for wells 28, 30 and 32. The dark center regions in these wells indicate flat center regions 46 where no positive reaction agglutination particles are reflecting or absorbing light. The light shined up from the bottom passes straight through the flat center section 46 and is not reflected back to the surface where the image to be digitized is focused thereby creating a dark spot in the image. For areas outside the center region 46, some of the scattered light is scattered back toward the surface on which the image to be digitized

is focused thereby creating a relatively lighter area in the image. This contrast can be detected as a negative reaction. A positive reaction will result in an image of the bottom which has a relatively uniform light intensity throughout the perimeter of the well bottom.

Referring to Figure 3B, there is shown an illustration of a U-shaped bottom. Again, agglutination particles such as the particle 54 are shown falling to the bottom of the well. This occurs under the influence of centrifugal force in a centrifuge. A difference between the U-shaped bottom and the terraced bottom is that when the agglutination particles reach the bottom of the well, they slide along the bottom until they reach the center during the spinning on the centrifuge. The particles 56 and 58 are shown sliding toward the center bottom of the well under the influence of centrifugal force. At the center bottom of the well, a "button" 62 of agglutinated material exists. This button 62 block or absorbs light in a beam 64 directed through the center bottom of the well in a conventional plate reader and forms dark spot in the center of a digitized image of the bottom of a well formed by a flat bed scanner. In contrast, a second light beam path shown at 66 directed off center through an area not occupied by the center button in a conventional plate reader passes through the well bottom and is bent but no substantial scattering occurs and no substantial light

absorption occurs because the light beam does not pass through the button 62. If no positive reaction occurred, the button 62 would not be formed, and both light beams 64 and 66 would experience approximately the same light absorption. Thus a plate reader of conventional design can distinguish between positive and negative reactions by comparing the light intensity values of the two beams 64 and 66. If a flat bed scanner is used, the digitized image of the bottom of a U-shaped well which has had a negative reaction will be substantially uniform in light intensity throughout the area of the bottom. Thus, suitable pattern recognition software can distinguish between positive and negative reactions by looking for a contrasting light intensity between the center area of the well and the surrounding areas.

Referring to Figures 4A and 4B, there is shown a flow chart of one typical pattern recognition computer program which could be used to implement the teachings of the method of the invention. The first step in the pattern recognition process is to determine what type of wells have been used in the assay. This step is symbolized by block 68. Step 68 represents the process of either reading a configuration file or prompting for and receiving user input from the keyboard as to whether U-shaped bottoms or terraced bottoms are in use. Next, in step 70, the digitized image data for the next well bottom

to be analyzed is retrieved from disk. In some embodiments multiple well bottom images may be simultaneously accessed and analyzed. In the preferred embodiment, only one well bottom is analyzed at a time.

Step 72 represents a test to determine whether the last well in a plate has been processed. If so, processing is vectored to a well pattern template matching routine which will be discussed later. The purpose of this template matching routine is to compare the pattern of positive and negative reactions to known patterns or "templates" to determine the results of an assay. If the well data retrieved in step 70 has not yet been analyzed, then processing is vectored to step 74 for decompression. Because it is more compact for storage purposes to encode the digitized image data using known encoding schemes, said encoding is done by the driver software for the flat bed scanner. Step 74 decodes the compressed data to reconstruct the pixel data representing the actual image as digitized.

The next step is to start the pattern recognition process of comparing light intensity values in various areas of the picture. This is symbolized by step 76. This step is the process of averaging the light intensity values of pixels in the center region of the image and comparing the average to the average light intensity of the pixels in the regions surrounding the

center region. How to define the size and shape of the center region may be defined in the configuration file or, in some embodiments, may be defined interactively by the user in real time on a reproduction of the well bottom image displayed on the monitor.

Next, processing branches to the proper subroutine to use the results of step 76 to decide whether the reaction was positive or negative. Since the conclusions are opposite depending upon which well bottom shape is in use, before the branching can occur, the decision as to which well bottom shape led to the image being processed must be made. This decision is made in step 78. This branching step looks at the data read from the configuration file to determine which well bottom shape created the image being processed and causes branching to step 80 if a terraced bottom was used and to step 82 if a U-shaped bottom shape was used.

Step 80 is a test to examine the results of the calculations performed in step 76 and to branch to the proper routine to label the well ID number with the reaction type depending upon the result. For terraced shaped bottoms, if there is a substantial difference between the average center section light intensity and the outer region light intensity, then the reaction is negative in the terraced bottom wells. The reaction is positive is there is no substantial difference between

these light intensity averages. Step 80 takes the two region averages and compares them for a substantial difference. Typically, this would be done by subtracting the two values and comparing the difference to a threshold constant which could be stored in the configuration file or, in some embodiments, could be supplied interactively by the user.

If a substantial difference is found by the test step 80, processing is vectored to step 83 where the well identification record in a data base having records for each well is updated. Each well record contains a field for the reaction type. This field is updated with data indicating the reaction type is negative if step 83 is reached. If no substantial difference is found in step 80, processing is vectored to step 84 where the well data base record is labeled with a positive reaction type data in the appropriate field. Processing then returns to step 70.

If processing in step 78 resulted in a transfer to step 82, the conclusions drawn will be exactly opposite as were drawn in step 80 since U-shaped bottoms look different. Step 82 again compares the center section average to the outer region average in the same way as was done in step 80. If a substantial difference is found between the center region average and the outside region average, then processing is vectored to step 86 where the

well identification record in the data base is updated to indicate a positive reaction occurred. If no substantial difference is found, step 88 is reached where the well record is updated to indicate a negative reaction occurred. Processing then returns to step 70 where the digitized data for the next well to be analyzed is retrieved from the hard disk.

Referring to Figure 5, there is shown a flow chart of the well pattern template matching routine to which processing is branched if test step 72 in Figure 4A determines that all wells have been processed. Step 90 is the first step in the process of template matching and involves accessing the well data base and retrieving the well reaction types for all wells having the same donor identification. Each well record in the data base has a field for the donor identification number of the donor whose red blood cells or plasma were deposited in the well. Each well record has a field that identifies the type of donor sample (red blood cells or plasma) that went into the well and the type of reagent that went into the well. All this data for all well records corresponding to a particular donor is retrieved in step 90.

Step 92 represents the process of comparing the reaction pattern of all the wells containing sample from the same donor to the template of reactions to the same group of reagents for known blood types. The types of

reagents used in the forward and back typing processes of blood grouping and typing assays and the pattern of positive and negative reactions to these various reagents is well known. In some embodiments, step 92 represents the process of comparing the well reaction pattern for the particular donor to all the known blood type templates, i.e., known patterns of positive and negative reactions to the same reagents. After this comparison, a determination is made as to whether any match occurred. In other embodiments, after each comparison a determination is made as to whether there was a match. If there was, then no further comparisons are made.

Of course the pattern recognition and template matching processes disclosed herein for blood typing and grouping assays are not the only use for the invention and the invention is not to be understood as limited to these types of assays. The invention includes methods and apparatus for performing other assays and work such as the sequencing of DNA where the assay or other operation results or can result in an image which has light areas and dark areas which contain the sought after information. Suitable pattern recognition programs which extract the information from the patterns of light and dark areas are to be understood as included within the scope of the invention. Thus, the invention has equal utility in reading the patterns of light and dark area on the images

of gels used to determine the sequence of nucleic acids in DNA or RNA. Other applications for the invention include any assay which creates an agglutination button such as antibody assays.

The next step is symbolized by test 94 where a determination is made which template, if any, matched the experimentally determined reaction pattern of the donor. If a match occurred, processing is transferred to step 96 where the blood type and group of the matching template is written to the blood type and group records of a data record in another data base of donor identification records.

If no match is found, processing proceeds to step 98 where the appropriate donor identification record in the donor data base is updated to indicate this donor is an NTD or no type determined.

After either step 96 or 98 is performed, a step 100 is performed to determine if all the donor samples for which test results have been determined have been processed against the known templates. If the answer is yes, processing stops. If the answer is no, processing is vectored back to step 90 to retrieve the reaction results for the next donor to be matched.

Although the invention has been described in terms of the preferred and alternative embodiments disclosed herein, those skilled in the art will appreciate

still other alternative embodiments which may be made without departing from the true spirit and scope of the invention. All such embodiments are intended to be included within the scope of the claims appended hereto.

WHAT IS CLAIMED IS:

1. An apparatus for analyzing patterns of light and dark areas in an image to extract information comprising:

means to digitize the image;

means to perform pattern recognition on the dark and light areas on the digital data representing said image to compare the pattern of light and dark area to known patterns to determine if there is a match and thereby extract information.

2. The apparatus of claim 1 wherein said means for digitizing comprises a flat bed scanner coupled to a computer.

3. The apparatus of claim 1 wherein said means for pattern recognition comprises a programmed computer.

4. The apparatus of claim 3 wherein said programmed computer further comprises means for computing the average light intensity of a predetermined region in the center of said image and further comprises means for computing the average light intensity of a predetermined region outside the center of said image and further comprises means for comparing the two averages to determine the degree of difference between said averages.

5. The apparatus of claim 4 further comprising means for drawing a conclusion from the difference of said averages and for recording that conclusion as said

information to be extracted from said pattern of light and dark areas.

6. The apparatus of claim 4 wherein said image is the image of the bottom of a test well in which has been performed a blood grouping or typing assay, said test well having any of a plurality of shapes for the well bottom with different optical characteristics and further comprising means in said means for comparing the average light intensity values for determining the well bottom shape and optical characteristics before extracting any information from the differences in said averages.

7. The apparatus of claim 6 further comprising means for drawing a conclusion from the differences in averages as to whether a positive or negative agglutination reaction to a test reagent occurred in a particular well and for recording the positive or negative result in a data base record corresponding to the particular well that created the image which was digitized.

8. The apparatus of claim 7 wherein the information to be extracted is blood type and group for the blood of a donor and wherein each of a plurality of wells contain aliquots of either red blood cells or plasma of a particular donor and wherein a first subset of said plurality of wells have placed therein a series of forward typing reagents and a second subset of said plurality of

wells have placed therein a series of back typing reagents, and wherein each said well in said plurality of wells has a data base record associated therewith with a field for the well identification number, a field for the donor identification number, and a field for the type of reagent which was placed in said well and further comprising means for recording the appropriate data in each of said fields in the data record for each well and further comprising means for comparing the pattern of positive and negative reactions in the wells containing sample aliquots from the blood of the same donor to known patterns of positive and negative reactions for known blood types and groups and for determining if there is or is not a match with any of the known patterns, and, if there is a match, for recording the blood type and group of the matching pattern as the blood type and group in a data record associated with this particular donor in a data base of such records, and, if there is no match, for recording in the data record for this donor a "no type determined" code in an appropriate field.

9. An apparatus for automatic analysis of blood typing and grouping assays to determine and record the blood type and group comprising:

means for digitizing the image of a plurality of well bottoms containing sample aliquots of donor blood;

means for computing the average light intensity values of the pixels located in a plurality of predetermined regions of said image;

means for comparing the average light intensities in the various regions to determine the differences;

means for drawing a conclusion from the differences in said averages as to whether each particular well had either a positive or a negative agglutination reaction and for recording these conclusions in data base records associated with the appropriate wells stored in a data base of such records;

means for extracting the pattern of positive and negative reactions to each type reagent for all the wells containing donor samples from the same donor;

means for comparing the pattern of positive and negative reactions for a particular donor to each of a plurality of known patterns of positive and negative reactions for known blood types and groups and for determining if any match exists.

10. A method of extracting information regarding chemical or biological assays or procedures comprising:

digitizing an image containing light and dark area containing the information sought by said assay or procedure; and

performing a pattern recognition process on said pattern of light and dark areas to compare the patterns of light and dark areas to known patterns of light and dark areas indicating certain conditions and drawing conclusions regarding said information to be extracted by the match or lack of match with any of the known patterns.

11. The method of claim 10 wherein said image is the image of the bottom of an assay well containing a reagent in a blood type and group assay and wherein said step of performing a pattern recognition process comprises the steps of computing the average light intensity in a plurality of predetermined regions of said image and comparing the averages so computed to determine the differences and comparing the differences to known patterns of differences for positive and negative agglutination reactions for the well type which created the image and drawing a conclusion as to whether the reaction was positive or negative based upon whether there was or was not a match with either of the known patterns.

12. The method of claim 11 further comprising the steps of performing said pattern recognition process on a plurality of wells each of which contains a sample aliquot from the same donors blood and a different one of a plurality of forward and reverse typing reagents and then comparing the pattern of positive and negative agglutination reactions to a set of known patterns of

positive and negative reactions to the same reagents for known blood types.

13. A method of automatically analyzing the results of blood typing and grouping assays to determine blood type and group comprising:

digitizing the images of the bottoms of a plurality of assay wells each of which contains a sample aliquot of red blood cells or blood plasma from a donor;

computing for each well bottom image the average light intensity in each of a plurality of predetermined regions;

computing the differences in average light intensity between various ones of said predetermined areas;

drawing a conclusion for each well from the differences previously computed as to whether the well experienced a positive or a negative reaction between the sample aliquot and the assay reagent contained therein;

recording the conclusion for each well in an appropriate filed of a data record corresponding to that well in a data base of such records for all the wells and recording the type of reagent that went into each well and the donor identification number for the donor from which the sample aliquot came for each well in appropriate fields of the corresponding data record for each well;

extracting the reagent type and reaction result for each well containing a sample aliquot from the same donor;

comparing the pattern of positive and negative reactions to the various forward and reverse typing reagents for the collection of wells for which data was extracted in the previous step to a plurality of known patterns of reaction results to the same reagents for a plurality of known blood types;

recording the blood type and group for the matching known pattern in the appropriate field of a data record associated with the donor if a match occurred between this donor's assay results and any of the known patterns from known blood types, and recording a code indication no blood type was determined in the appropriate field of the corresponding data record for the donor when no match occurs.

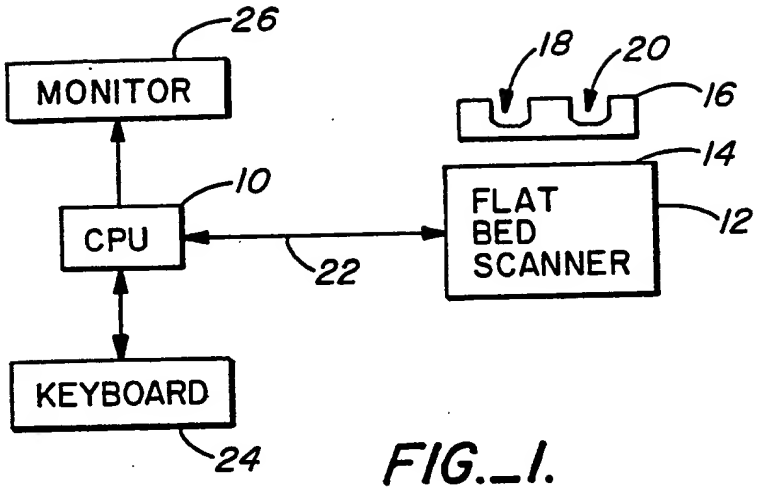
26
MONITOR
18
20
16
14
10
CPU
FLAT
BED
SCANNER
12
22
KEYBOARD
24

FIG._1.

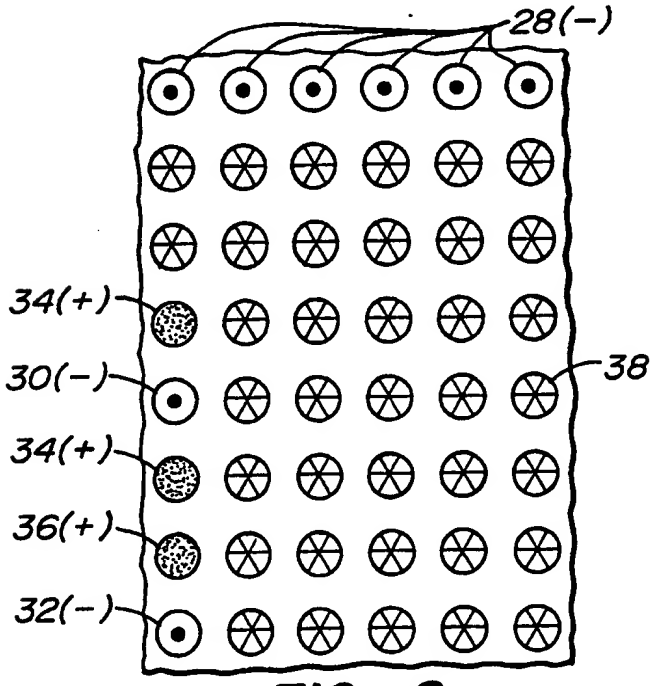
28(-)
34(+)
30(-)
34(+)
36(+)
32(-)
38

FIG._2.

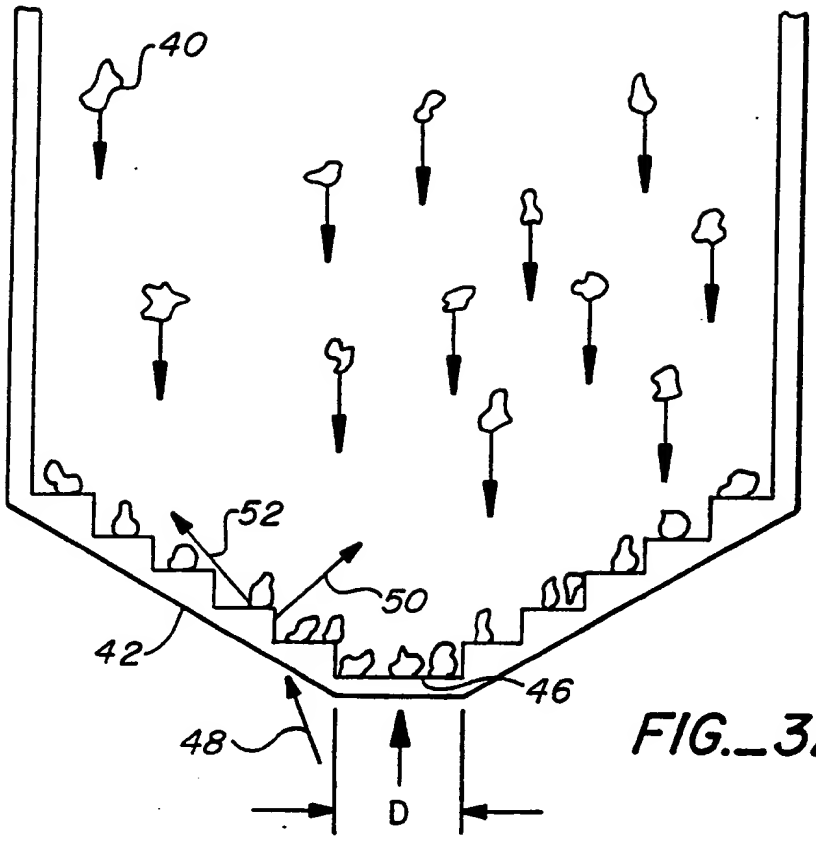


FIG._3A.

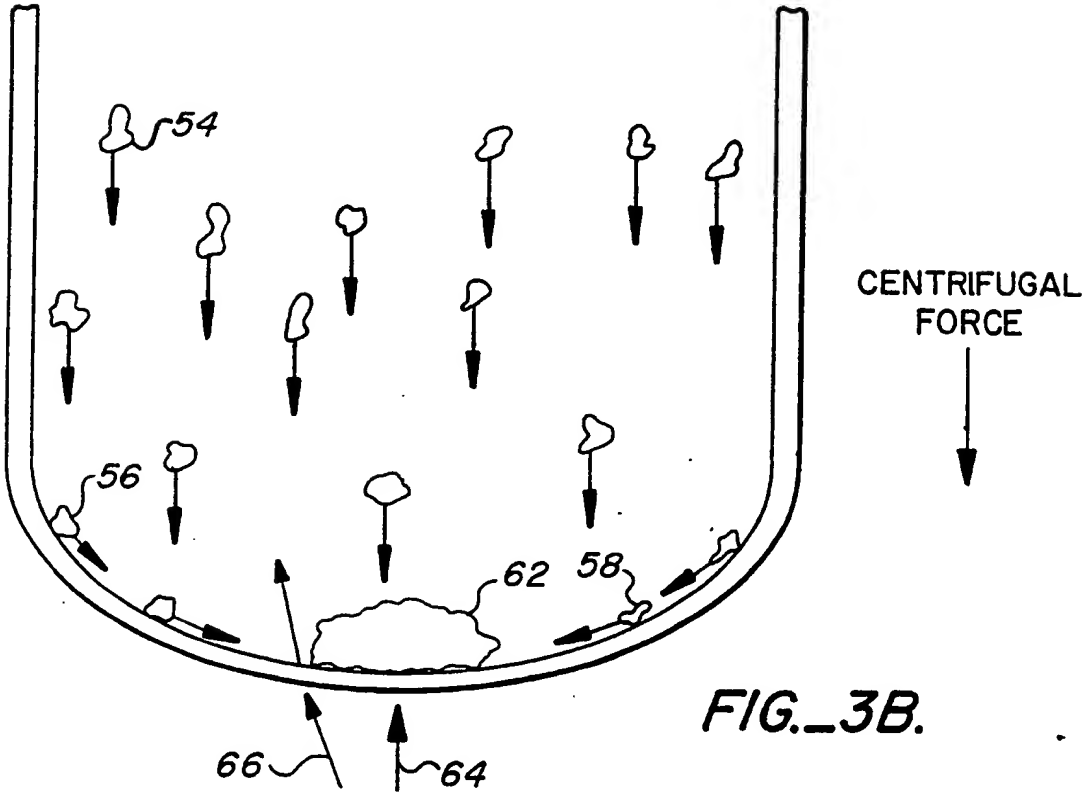


FIG._3B.

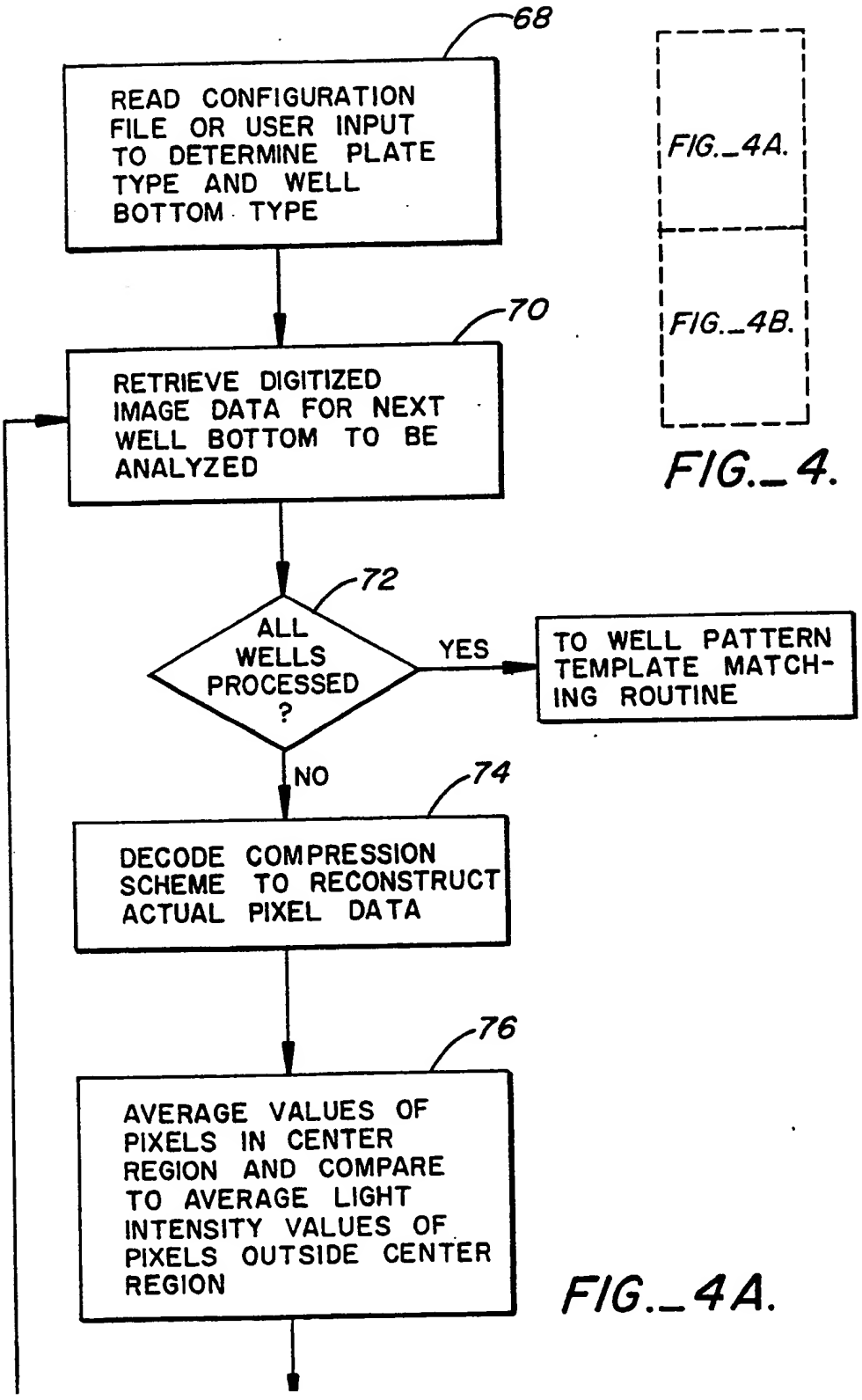


FIG._4A.

FIG._4B.

FIG._4.

FIG._4A.

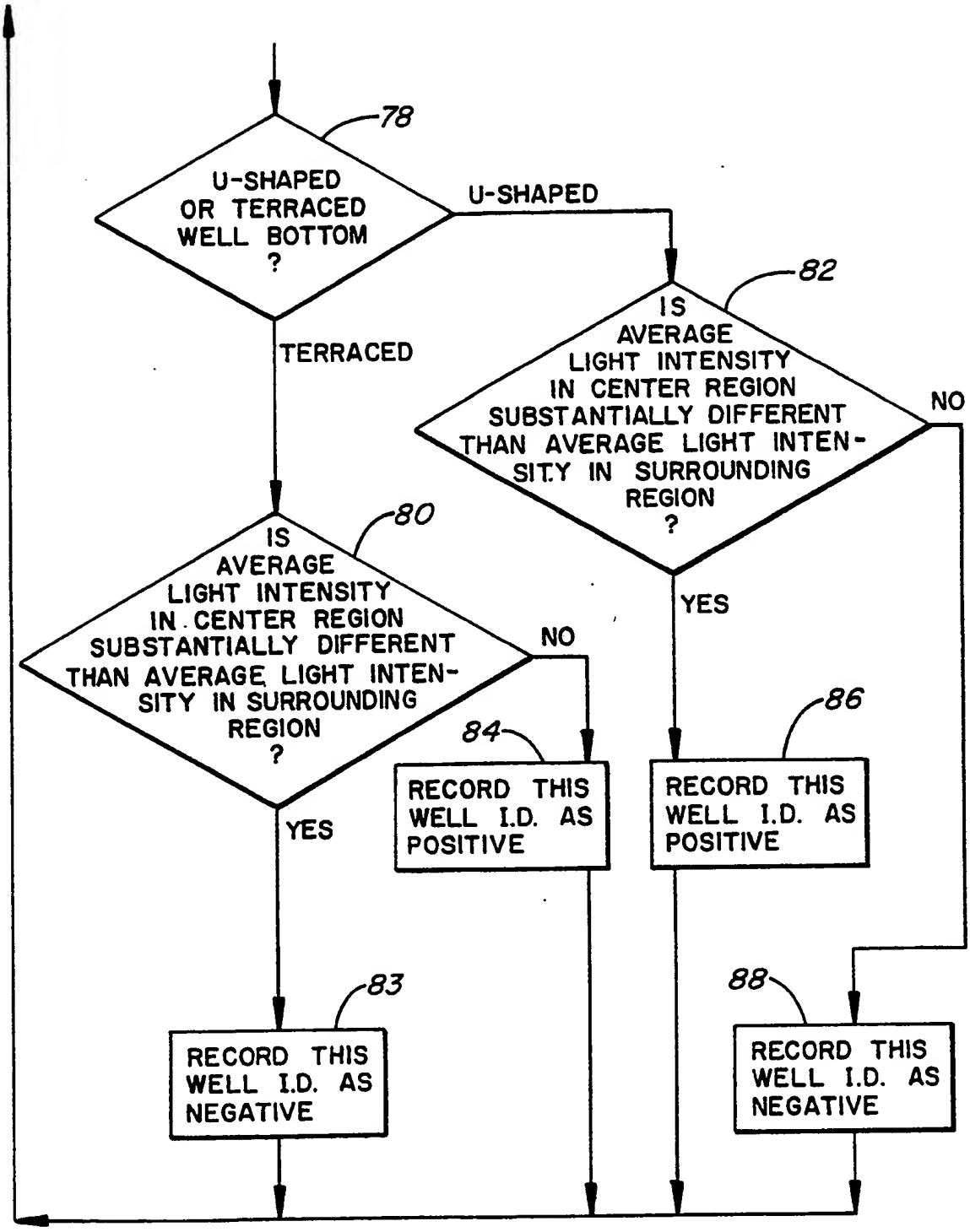
78
U-SHAPED OR TERRACED WELL BOTTOM ?
U-SHAPED
TERRACED
82
IS AVERAGE LIGHT INTENSITY IN CENTER REGION SUBSTANTIALLY DIFFERENT THAN AVERAGE LIGHT INTENSITY IN SURROUNDING REGION ?
NO
YES
80
IS AVERAGE LIGHT INTENSITY IN CENTER REGION SUBSTANTIALLY DIFFERENT THAN AVERAGE LIGHT INTENSITY IN SURROUNDING REGION ?
NO
YES
84
RECORD THIS WELL I.D. AS POSITIVE
86
RECORD THIS WELL I.D. AS POSITIVE
83
RECORD THIS WELL I.D. AS NEGATIVE
88
RECORD THIS WELL I.D. AS NEGATIVE

FIG._4B.

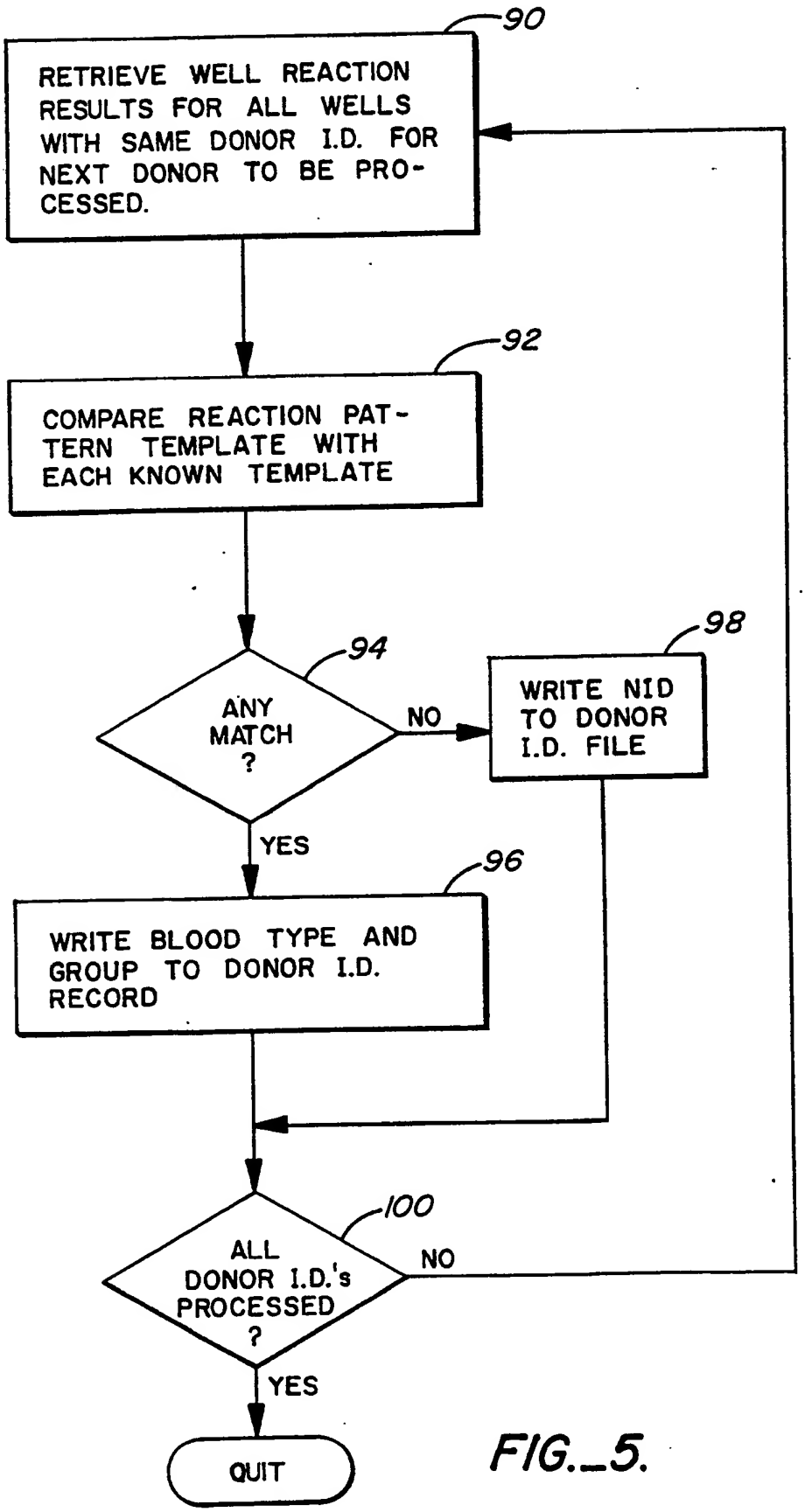
90
RETRIEVE WELL REACTION RESULTS FOR ALL WELLS WITH SAME DONOR I.D. FOR NEXT DONOR TO BE PROCESSED.
92
COMPARE REACTION PATTERN TEMPLATE WITH EACH KNOWN TEMPLATE
94
ANY MATCH ?
NO
98
WRITE NID TO DONOR I.D. FILE
YES
96
WRITE BLOOD TYPE AND GROUP TO DONOR I.D. RECORD
100
ALL DONOR I.D.'s PROCESSED ?
NO
YES
QUIT

FIG._5.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/00296

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: G 01 N 21/82; G 01 N 33/80

**II. FIELDS SEARCHED**

**Minimum Documentation Searched [7]**

| Classification System | Classification Symbols |
|---|---|
| IPC[4] | G 01 N 21/82; G 01 N 27/26; G 06 K 9/64; G 01 N 33/80 |

**Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]**

**III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]**

| Category [10] | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No. [13] |
|---|---|---|
| X | Optical Engineering, vol. 26, no. 7, July 1987, (Bellingham, WA, US), S.B. Serpico et al.: "Problems and prospects in image processing of two-dimensional gel electrophoresis", pages 661-668 see pages 661-662, part 1."Introduction"; figure 1; pages 664-665, part 3. "Feature extraction and clustering"; part 4. "Matching of electrophoresis images" -- | 1,10 |
| X | EP, A, 0079717 (OLYMPUS OPTICAL CO.) 25 May 1983 see page 7, lines 19-26; pages 8,9; page 10, lines 1-23 -- | 1-3,6,8, 9-13 |

./.

[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 23rd May 1989 | 14 JUIN 1989 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUROPEAN PATENT OFFICE | P.C.G. VAN DER POTTEN |

International Application No. PCT/US 89/00296

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No |
|---|---|---|
| X | Pattern Recognition Letters, vol. 5, no. 1, January 1987, Elsevier Science Publishers B.V. (North-Holland), (Amsterdam, NL), H.H.-S. Ip et al.: "Comparison of 2-D gel electrophoresis images", pages 81-86 see page 81, part 1."Introduction"; page 84, part 6 - part 6.1., "Implementation" --- | 1,10 |

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8900296 SA 26814

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 09/06/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP-A- 0079717 | 25-05-83 | JP-A- 58095248 | 06-06-83 |
| | | US-A- 4556641 | 03-12-85 |